Transparency Declaration

The authors have no conflicts of interest to declare.

References

1. Thong K, Lim K, Yeo C, Puthucheary S, Yasin R. Genotypic characterization of extended-spectrum beta-lactamases producing *Klebsiella pneumoniae* strains isolated in Malaysia. *Int J Infect Dis* 2008; 12: E117.
2. Poirel L, Naas T, Nordmann P. Genetic support of extended-spectrum beta-lactamases. *Clin Microbiol Infect* 2008; 1: 75–81.
3. Yan J, Ko W, Wu J. Identification of a plasmid encoding SHV-12, TEM-1, and a variant of IMP-2 metallo-beta-lactamase, IMP-8, from a clinical isolate of *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2001; 45: 2368–2371.
4. Toleman M, Bennett P, Walsh T. IS*CR* elements: novel gene-capturing systems of the 21st century? *Microbiol Mol Biol Rev* 2006; 70: 296–316.
5. Partridge S, Hall R. In34, a complex In5 family class 1 integron containing *orf513* and *dfrA10*. *Antimicrob Agents Chemother* 2003; 47: 342–349.
6. Quiroga M, Andres P, Petroni A *et al.* Complex class 1 integrons with diverse variable regions, including *aac(6′)-Ib-cr*, and a novel allele, *qnrB*10, associated with IS*CR1* in clinical enterobacterial isolates from Argentina. *Antimicrob Agents Chemother* 2007; 51: 4466–4470.
7. Toleman M, Bennett P, Walsh T. Common regions e.g. *orf513* and antibiotic resistance: IS91-like elements evolving complex class 1 integrons. *J Antimicrob Chemother* 2006; 58: 1–6.
8. Chang C, Fang Y, Tsai S, Chang L, Yu W. Characterization of class 1 integrons and gene cassettes in clinical isolates of *Klebsiella pneumoniae* from Taiwan. *Diagn Microbiol Infect Dis* 2009; 65: 214–216.
9. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests. Approved Standard, Ninth Edition. 2006: CLSI, Wayne, PA, USA.
10. Wei Q, Yang Z, Chen N, Chen X, Li G, Lu Y. *dfrA27*, a new integron-associated trimethoprim resistance gene from *Escherichia coli*. *J Antimicrob Chemother* 2009; 63: 405–406.
11. Wang M, Tran J, Jacoby G, Zhang Y, Wang F, Hooper D. Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Shanghai, China. *Antimicrob Agents Chemother* 2003; 47: 2242–2248.
12. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins K, Threlfall E. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005; 63: 219–228.
13. Lee M, Peng C, Hsu H, Chen Y. Molecular characterization of the metallo-beta-lactamase genes in imipenem-resistant Gram-negative bacteria from a university hospital in southern Taiwan. *Int J Antimicrob Agents* 2008; 32: 475–480.
14. Wang A, Yang Y, Lu Q *et al.* Presence of *qnr* gene in *Escherichia coli* and *Klebsiella pneumoniae* resistant to ciprofloxacin isolated from pediatric patients in China. *BMC Infect Dis* 2008; 8: 68 (1–6).
15. Barlow R, Pemberton J, Desmarchelier P, Gobius K. Isolation and characterization of integron-containing bacteria without antibiotic selection. *Antimicrob Agents Chemother* 2004; 48: 838–842.
16. Bae I, Lee Y, Lee W, Lee S, Jeong S. Novel complex class 1 integron bearing an IS*CR1* element in an *Escherichia coli* isolate carrying the $bla_{CTX\text{-}M\text{-}14}$ gene. *Antimicrob Agents Chemother* 2007; 51: 3017–3019.
17. Toleman M, Bennett P, Jones R, Walsh T. Global emergence of trimethoprim/sulfamethoxazole resistance in *Stenotrophomonas maltophilia* mediated by acquisition of *sul* genes. *Emerg Infect Dis* 2007; 13: 559–565.
18. Henriques I, Fonseca F, Alves A, Saavedra M, Correia A. Occurrence and diversity of integrons and beta-lactamase genes among ampicillin-resistant isolates from estuarine waters. *Res Microbiol* 2006; 157: 938–947.
19. Wu J, Ko W, Tsai S, Yan J. Prevalence of plasmid-mediated quinolone resistance determinants *qnrA*, *qnrB*, and *qnrS* among clinical isolates of *Enterobacter cloacae* in a Taiwanese hospital. *Antimicrob Agents Chemother* 2007; 51: 1223–1227.
20. Weisburg W, Barns S, Pelletier D, Lane D. 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 1991; 173: 697–703.

Molecular characteristics of extended-spectrum β-lactamase-producing *Escherichia coli* from Rio de Janeiro, Brazil

G. Peirano[1,2], M. D. Asensi[3], A. Pitondo-Silva[4] and J. D. D. Pitout[1,2,5]

1) *Division of Microbiology, Calgary Laboratory Services,* 2) *Department of Pathology & Laboratory Medicine, University of Calgary, Calgary, AB, Canada,* 3) *Laboratório de Pesquisas em Infecção Hospitalar, Oswaldo Cruz Institute, Rio de Janeiro, RJ,* 4) *Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Universidade de São Paulo, Ribeirão Preto, SP, Brazil and* 5) *Microbiology and Infectious Diseases, University of Calgary, Calgary, AB, Canada*

Abstract

Twenty-five extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* clinical isolates from Rio de Janeiro, Brazil were characterized by isoelectric focusing, PCR and sequencing of bla_{ESBL} genes, plasmid-mediated quinolone resistance determinants, phylogenetic groups, replicon typing, pulsed-field electrophoresis, and multilocus sequencing typing. Twenty-three (92%) ESBL-producing *E. coli* isolates were positive for $bla_{CTX\text{-}M}$ genes, *aac(6′)-Ib-cr*, and *qnrB*. Genetic relatedness of ESBL producers clustered seven (28%) CTX-M-15-producing isolates as sequence type (ST)410, clonal complex (CC) 23, and two (8%) as clone O25-ST131. Our results illustrate the predominance of phylogroup A (52%), ST410 (CC 23) and CTX-M-15 among ESBL-producing *E. coli* isolates from hospitals in Rio de Janeiro.

Keywords: Brazil, CTX-M-15, *Escherichia coli*, phylogroup A, ST410

Original Submission: 27 August 2010; **Revised Submission:** 9 November 2010; **Accepted:** 28 November 2010
Edited: R. Cantón
Article published online: 4 December 2010
Clin Microbiol Infect 2011; **17:** 1039–1043
10.1111/j.1469-0691.2010.03440.x

Corresponding author: J. D. D. Pitout, Calgary Laboratory Services, #9, 3535 Research Road NW, Calgary, Alberta T2L 2K8, Canada
E-mail: johann.pitout@cls.ab.ca

Extended-spectrum β-lactamase (ESBL) production is a common mechanism of antimicrobial resistance among Gram-negative bacteria. ESBL-producing *Escherichia coli* strains cause several types of infection, including urinary tract infections, and are associated with increased patient morbidity and mortality [1]. CTX-M-type enzymes are the most prevalent ESBLs detected on a worldwide basis, mainly in *E. coli*. CTX-M β-lactamases include more than 80 different enzymes, clustered into five groups: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25 [2]. Recently, a particular clone of CTX-M-15-producing *E. coli* identified as sequence type (ST)131 by multilocus sequence typing (MLST) has been identified from several countries [3]. The ST131 clone is characterized by belonging to the highly virulent phylogenetic type B2, serotype O25:H4, and harbouring multidrug-resistant IncFII plasmids, and appears to be responsible for a large proportion of the international epidemic of CTX-M-producing *E. coli* [4]. In Brazil, ESBL-producing *Enterobacteriaceae* have been increasingly recovered from both hospitals [5–7] and community settings [8, 9] as important causes of bacteraemia and urinary tract infections.

The present study was undertaken to characterize ESBL-producing *E. coli* isolates collected from public hospitals in Rio de Janeiro. Twenty-five non-duplicate β-lactam-resistant *E. coli* isolates were retained for study from both inpatients (n = 16) and outpatients (n = 9) in hospitals located in the Rio de Janeiro metropolitan area, Brazil, from February 2008 to June 2009. Isolates were recovered from blood (n = 8), urine (n = 8), rectal swabs (n = 6), ascitic fluid (n = 2) and wound (n = 1) cultures. ESBL production was detected according to the 2009 CLSI guidelines, i.e. screening with ceftriaxone and ceftazidime at 1 mg/L, respectively, and confirming with ceftazidime, ceftazidime/clavulanate, cefotaxime and cefotaxime/clavulanate disks [10]. Antimicrobial susceptibility testing was determined with the VITEK 2 instrument (Vitek AMS; bioMerieux Vitek Systems, Hazelwood, MO, USA). The MICs of the following drugs were determined with a custom-made Gram-negative card (AST-N121): piperacillin–tazobactam (concentrations (mg/L), 4/4, 16/4, 32/4 and 64/4), ertapenem (concentrations (m/L), 0.5, 1 and 4), meropenem (concentrations (mg/L), 0.5, 4 and 16), ciprofloxacin (concentrations (mg/L), 0.5, 2 and 4), gentamicin (concentrations (mg/L), 4, 16 and 32), trimethoprim–sulphamethoxazole (concentrations (mg/L), 1/19, 4/76 and 16/304), amoxycillin–clavulanic acid (concentrations (mg/L), 4/2, 16/8 and 32/16), nitrofurantoin (concentrations (mg/L), 16, 32 and 64), amikacin (concentrations (mg/L), 8, 16 and 64), tobramycin (concentrations (mg/L), 8, 16 and 64), and cefoxitin (concentrations (mg/L), 8, 16 and 32). Throughout this study, results were interpreted according to CLSI criteria [10]. Of the 25 isolates, 24 (96%) were non-susceptible (i.e. intermediate or resistant) to trimethoprim–sulphamethoxazole, 23 (92%) to ciprofloxacin and amoxycillin–clavulanic acid, 20 (80%) to tobramycin, 15 (60%) to gentamicin, seven (28%) to piperacillin–tazobactam and cefoxitin, six (24%) to amikacin, and three (12%) to nitrofurantoin. The MICs of ceftriaxone ranged from 8 to >32 mg/mL, those of ceftazidime from 1 to >32 mg/L, and and those of cefepime from 2 to >32 mg/L.

ESBLs were identified as previously reported [11]. Twenty-three isolates (92%) were positive for $bla_{\text{CTX-M}}$ genes: 16 produced CTX-M-15, three produced CTX-M-8, two produced CTX-M-3, and two produced CTX-M-2, whereas the remaining two isolates produced SHV-2. Some of the CTX-M-producing isolates also produced TEM-1 and OXA-1 (i.e. those with CTX-M-3 and CTX-M-15) β-lactamases (Table 1).

Amplification of *qnrA*, *qnrB* and *qnrS* was undertaken in all CTX-M-positive isolates by multiplex PCR [12]. The *aac(6′)-Ib-cr* and *qepA* genes were amplified in a separate PCR [12,13]. Eleven (44%) of the ESBL-producing *E. coli* isolates were positive for *aac(6′)-Ib-cr*, four (16%) were positive for both *qnrB* and *aac(6′)-Ib-cr*, and one was positivefor *qnrB* (Table 1).

The clonal relationship between CTX-M producers was determined by pulsed-field gel electrophoresis (PFGE) and MLST. All ESBL-producing isolates were screened for clone O25-ST131 with a PCR for the *pabB* allele [14]. Phylogenetic analyses were performed with the algorithm eBUSRT V3 [15]. Overall, 16 isolates were considered to be non-related by PFGE, indicating that ESBL-producing *E. coli* isolates constituted a relatively diverse population. Only one PFGE cluster (comprising seven isolates) was clearly identified among the 23 typeable *E. coli* isolates (Fig. 1). Nevertheless, isolates of this clonal group shared <85% similarity by PFGE, were identified as ST410, and belonged to clonal complex (CC)23 according to MLST (Fig. 2). Two isolates were confirmed to

TABLE 1 Characteristics of extended-spectrum β-lactamase-producing *Escherichia coli* isolates in Rio de Janeiro

Isolate no.	Specimen	Antimicrobial non-susceptibility[a]	PMQR	β-Lactamases	Phylogenetic group
BRA 3912	Blood	TZP, CIP, SXT, AMC, AMK, TOB	*aac(6′)-Ib-cr*	CTX-M-15	A
BRA 3995	Blood	CIP, NIT	–	CTX-M-8	B1
BRA 4074	Urine	CIP, SXT, AMC	–	CTX-M-2	A
BRA 4118	Blood	TZP, CIP, GEN, SXT, AMC, AMK, TOB, FOX	*aac(6′)-Ib-cr/qnrB*	CTX-M-15	B2
BRA 4134	Ascitic fluid	SXT, AMC, TOB	*aac(6′)-Ib-cr*	TEM-1, OXA-1, CTX-M-15	A
BRA 4141	Urine	CIP, SXT, AMC, NIT, TOB, FOX	*aac(6′)-Ib-cr*	CTX-M-15	A
BRA 4147	Urine	SXT, AMC	–	CTX-M-2	B1
BRA 4155	Blood	CIP, GEN, SXT, AMC, TOB	*aac(6′)-Ib-cr*	TEM-1, OXA-1, CTX-M-15	B2
BRA 4168	Urine	CIP, GEN, SXT, AMC, TOB	*aac(6′)-Ib-cr*	TEM-1, OXA-1, CTX-M-15	A
BRA 4225	Blood	CIP, SXT, AMC, AMK, TOB	*aac(6′)-Ib-cr*	TEM-1, CTX-M-15	A
BRA 4318	Ascitic fluid	TZP, CIP, GEN, SXT, AMC, AMK, TOB	*aac(6′)-Ib-cr/qnrB*	TEM-1, OXA-1, CTX-M-15	B1
BRA 4334	Urine	TZP, CIP, GEN, SXT, AMC, TOB	–	TEM-1, OXA-1, CTX-M-15	B2
BRA 4374	Blood	CIP, GEN, SXT, TOB	–	SHV-2	D
BRA 4417	Urine	TZP, CIP, GEN, SXT, AMC, TOB, FOX	*aac(6′)-Ib-cr*	TEM-1, OXA-1, CTX-M-3	B2
BRA 4422	Wound	CIP, SXT, AMC, FOX	–	OXA-1, CTX-M-15	A
BRA 4631	Blood	CIP, GEN, SXT, AMC, TOB, FOX	*aac(6′)-Ib-cr/qnrB*	TEM-1, CTX-M-15	B1
BRA 4718	Urine	CIP, GEN, SXT, AMC, TOB	–	SHV-2	A
BRA 4771	Blood	CIP, GEN, SXT, AMC, TOB	*aac(6′)-Ib-cr*	TEM-1, OXA-1, CTX-M-15	A
BRA 4839	Urine	CIP, GEN, SXT, AMC, TOB, FOX	–	TEM-1, OXA-1, CTX-M-3	D
BRA 4842	Rectal swab	CIP, SXT, AMC, TOB	*aac(6′)-Ib*	CTX-M-8	B1
BRA 4857	Rectal swab	SXT, AMC	*qnrB*	CTX-M-8	A
BRA 4861	Rectal swab	CIP, GEN, SXT, AMC, NIT, AMK, TOB	*aac(6′)-Ib-cr*	TEM-1, OXA-1, CTX-M-15	A
BRA 4991	Rectal swab	CIP, GEN, SXT, AMC, TOB	*aac(6′)-Ib-cr*	TEM-1, OXA-1, CTX-M-15	A
BRA 4993	Rectal swab	CIP, GEN, SXT, AMC, TOB	*aac(6′)-Ib-cr*	OXA-1, CTX-M-15	A
BRA 4998	Rectal swab	TZP, CIP, GEN, SXT, AMC, AMK, TOB, FOX	*aac(6′)-Ib-cr/qnrB*	TEM-1, OXA-1, CTX-M-15	D

AMC, amoxycillin–clavulanic acid; AMK, amikacin; CIP, ciprofloxacin; FOX, cefoxitin; GEN, gentamicin; NIT, nitrofurantoin; PMQR, plasmid-mediated quinolone resistance determinant; SXT, trimethoprim–sulphamethoxazole; TOB, tobramycin; TZP, piperacillin/tazobactam..
[a]Antimicrobial non-susceptibility (i.e. resistant or intermediate).

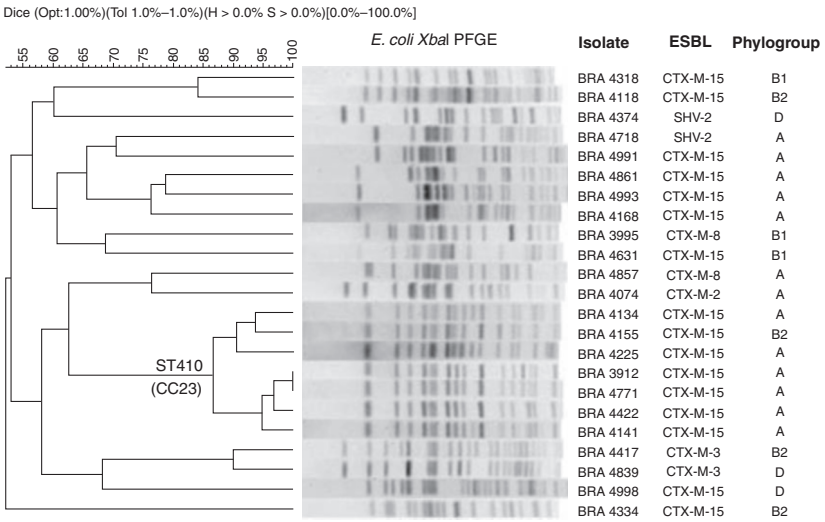

FIG. 1. Pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested DNA of 23 extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates from Rio de Janeiro.

be ST131 with PCR for the *pabB* allele and MLST, but they did not cluster together by PFGE.

The ESBL-producing *E. coli* isolates were assigned to phylogenetic groups A (n = 13), B1 (n = 5), B2 (n = 4) and D (n = 3) by multiplex PCR [16]. Phylogroup B2 comprised the two ST131 isolates and also two non-ST131 CTX-M-15-producing isolates. All but one ST410 isolate belonged to phylogenetic group A.

Plasmid sizes were determined with the use of previously described protocols and conditions [11], and assigned to plasmid families by PCR-based replicon typing [17]. Conjugation experiments were performed by mating-out assays with a selection agar containing ceftriaxone 1 mg/L and *E. coli* C600N as recipient. The plasmid sizes in the CTX-M producers ranged from 80 to 135 kb, and belonged to the narrow host range incompatibility group IncFII, and the FIA and

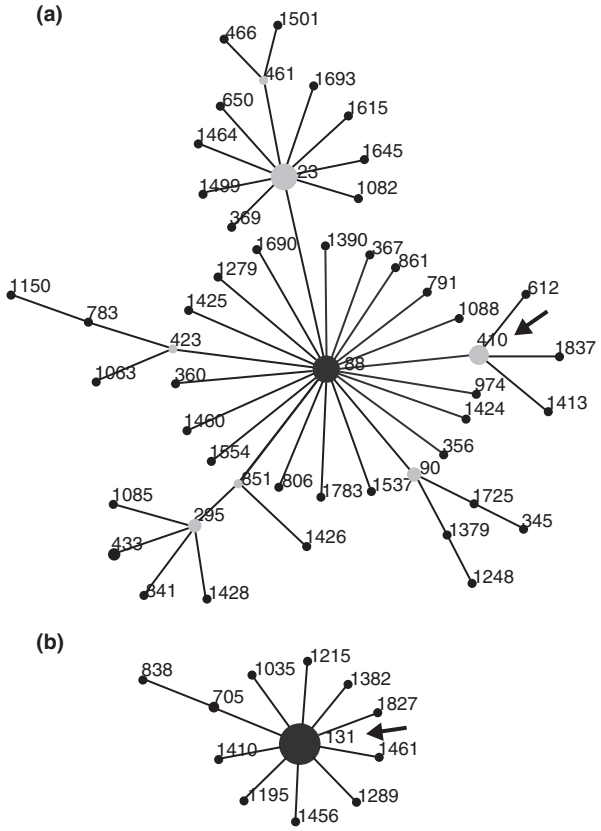

FIG. 2. Application of the eBURST algorithm to multilocus sequence typing data, showing clonal complex 23 and the respective sequence type (ST410) (a) and the ST131 group (b) of extended-spectrum β-lactamase-producing *Escherichia coli* isolates from Rio de Janeiro. Each sequence type is represented by a dot, and the size of each one is proportional to the number of *E. coli* isolates of each sequence type, considering all isolates in the database. Sequence types found in this study are indicated by an arrow.

FIB (18 isolates), P and B/O (one isolate), FIB (three isolates) and repF (one isolate) replicons were detected.

In the present study, CTX-M-15 enzymes were the most common ESBLs detected; the isolates producing these were multiresistant, and co-produced TEM-1 and OXA-1 β-lactamases; the majority were positive for *aac(6′)-Ib-cr*, and belonged to phylogenetic group A. PFGE showed one dominant clone and MLST analysis identified it as ST410 (CC23), which belonged to phylogenetic group A. This was the first detection of ST410 CC23 in Brazil, where recent MLST studies showed high genetic variability among *E. coli* isolates [18,19]. The detection of ST410/phylogroup A has so far been described in one CTX-M-14-producing *E. coli* isolate from Spain [20].

Our study also detected *aac(6′)-Ib-cr* among isolates from Brazil, highlighting the prevalence of this plasmid-mediated quinolone resistance gene, and indicates that there is widespread dissemination of *aac(6′)-Ib-cr*, particularly associated with CTX-M-producing isolates.

In conclusion, this study constitutes the first report of ST410 (CC23) isolates producing CTX-M-15 with the association of *aac(6′)-Ib-cr* as a plasmid-mediated quinolone resistance gene in Brazil.

Acknowledgements

This work was presented at the 50th Interscience Conference on Antimicrobial Agents and Chemotherapy, Boston, USA in 2010. This work was supported by research grants from the Calgary Laboratory Services (#73-4063) and from Merck (Canada).

Transparency Declaration

JDDP has previously received research funds from Merck and Astra Zeneca. All other authors: none to declare.

References

1. Oteo J, Pérez-Vázquez M, Campos J. Extended-spectrum [beta]-lactamase producing *Escherichia coli*: changing epidemiology and clinical impact. *Curr Opin Infect Dis* 2010; 23: 320–326.
2. Pitout JD, Laupland KB. Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. *Lancet Infect Dis* 2008; 8: 159–166.
3. Nicolas-Chanoine MH, Blanco J, Leflon-Guibout V *et al.* Intercontinental emergence of *Escherichia coli* clone O25:H4-ST131 producing CTX-M-15. *J Antimicrob Chemother* 2008; 61: 273–281.
4. Peirano G, Pitout JD. Molecular epidemiology of *Escherichia coli* producing CTX-M beta-lactamases: the worldwide emergence of clone ST131 O25:H4. *Int J Antimicrob Agents* 2010; 35: 316–321.
5. Silva Dias RC, Borges-Neto AA, D'Almeida Ferraiuoli GI, de-Oliveira MP, Riley LW, Moreira BM. Prevalence of AmpC and other beta-lactamases in enterobacteria at a large urban university hospital in Brazil. *Diagn Microbiol Infect Dis* 2008; 60: 79–87.
6. Dropa M, Balsalobre LC, Lincopan N *et al.* Extended-spectrum beta-lactamases among Enterobacteriaceae isolated in a public hospital in Brazil. *Rev Inst Med Trop Sao Paulo* 2009; 51: 203–209.
7. Oliveira CF, Dal Forno NL, Alves IA, Horta JA, Rieger A, Alves SH. Prevalence of the TEM, SHV and CTX-M families of extended-spectrum beta-lactamases in *Escherichia coli* and *Klebsiella* spp at the University Hospital of Santa Maria, State of Rio Grande do Sul. *Rev Soc Bras Med Trop* 2009; 42: 556–560.
8. Minarini LA, Poirel L, Trevisani NA, Darini AL, Nordmann P. Predominance of CTX-M-type extended-spectrum beta-lactamase genes among enterobacterial isolates from outpatients in Brazil. *Diagn Microbiol Infect Dis* 2009; 65: 202–206.
9. Dias RC, Marangoni DV, Smith SP *et al.* Clonal composition of *Escherichia coli* causing community-acquired urinary tract infections in the State of Rio de Janeiro, Brazil. *Microb Drug Resist* 2009; 15: 303–308.

10. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing; 19th informational supplement M100-S19*. Wayne, PA: CLSI, 2009.
11. Pitout JD, Church DL, Gregson DB *et al.* Molecular epidemiology of CTX-M-producing *Escherichia coli* in the Calgary Health Region: emergence of CTX-M-15-producing isolates. *Antimicrob Agents Chemother* 2007; 51: 1281–1286.
12. Pitout JD, Wei Y, Church DL, Gregson DB. Surveillance for plasmid-mediated quinolone resistance determinants in Enterobacteriaceae within the Calgary Health Region, Canada: the emergence of *aac(6′)-Ib-cr*. *J Antimicrob Chemother* 2008; 61: 999–1002.
13. Yamane K, Wachino J, Suzuki S, Arakawa Y. Plasmid-mediated *qepA* gene among *Escherichia coli* clinical isolates from Japan. *Antimicrob Agents Chemother* 2008; 52: 1564–1566.
14. Clermont O, Dhanji H, Upton M *et al.* Rapid detection of the O25b-ST131 clone of *Escherichia coli* encompassing the CTX-M-15-producing strains. *J Antimicrob Chemother* 2009; 64: 274–277.
15. Feil EJ, Li BC, Aanensen DM, Hanage WP, Spratt BG. eBURST:inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. *J Bacteriol* 2004; 186: 1518–1530.
16. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol* 2000; 66: 4555–4558.
17. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005; 63: 219–228.
18. Pitondo-Silva A, Minarini LAR, Camargo ILBC, Darini ALC. Clonal relationships determined by multilocus sequence typing among enteropathogenic *Escherichia coli* isolated in Brazil. *Can J Microbiol* 2009; 55: 672–679.
19. Minarini LAR, Camargo ILBC, Pitondo-Silva A, Darini ALC. Multilocus sequence typing of uropathogenic ESBL-producing *Escherichia coli* isolated in a Brazilian community. *Curr Microbiol* 2007; 55: 524–529.
20. Oteo J, Diestra K, Juan C *et al.* Extended-spectrum beta-lactamase-producing *Escherichia coli* in Spain belong to a large variety of multilocus sequence typing types, including ST10 complex/A, ST23 complex/A and ST131/B2. *Int J Antimicrob Agents* 2009; 34: 173–176.

Phenotypic identification of over 1000 isolates of anaerobic bacteria recovered between 1999 and 2008 in a major Costa Rican hospital

E. Rodríguez-Cavallini, P. Vargas, C. Rodríguez, C. Quesada-Gómez and M.-M. Gamboa-Coronado

Laboratorio de Investigación en Bacteriología Anaerobia (LIBA), Centro de Investigación en Enfermedades Tropicales (CIET), and Facultad de Microbiología, Universidad de Costa Rica, Ciudad Universitaria Rodrigo Facio, San José, Costa Rica

Abstract

Because of limitations in infrastructure, the aetiology of infections caused by anaerobic bacteria is seldom determined in clinical laboratories of developing countries. This study reports on the identification of 1010 anaerobic bacterial isolates collected between 1999 and 2008 in a major Costa Rican hospital with the use of two commercial phenotypic systems (RapID 32A and API 20A). Approximately 60% of the isolates were Gram-positive and, among the 35 species of Gram-positive bacteria found, the genera *Clostridium*, *Propionibacterium* and *Eggerthella*, and anaerobic cocci predominated. Twenty eight species were found among 395 isolates of Gram-negative bacteria. Species of *Bacteroides* were very frequent, followed by species of *Prevotella*, *Veillonella*, *Fusobacterium* and *Porphyromonas*.

Keywords: Anaerobic bacteria, biochemical identification, clinical samples, Costa Rica

Original Submission: 16 September 2010; **Revised Submission:** 13 October 2010; **Accepted:** 28 October 2010
Editor: D. Raoult
Article published online: 3 November 2010
Clin Microbiol Infect 2011; **17:** 1043–1047
10.1111/j.1469-0691.2010.03419.x

Corresponding author: C. Rodríguez, Laboratorio de Investigación en Bacteriología Anaerobia (LIBA), Centro de Investigación en Enfermedades Tropicales (CIET), and Facultad de Microbiología, Universidad de Costa Rica, Ciudad Universitaria Rodrigo Facio, San Pedro de Montes de Oca 2060, San José, Costa Rica
E-mail: cesar.rodriguezsanchez@ucr.ac.cr

Numerous species of anaerobic bacteria proliferate in the surfaces and cavities that make up the human body. This anaerobic microbiota is innocuous under usual conditions; however, it can cause pathology in the head and neck area, the thorax, the abdomen, the skin and soft tissues or the urogenital tract when certain predisposing conditions are present, such as compromised integrity of the skin or mucosae, necrosis resulting from ischaemic processes or the formation of abscesses [1].

The majority of clinical laboratories in Costa Rica and in other developing countries lack the infrastructure and experience required to isolate and identify anaerobic bacteria [2]. Such an absence of knowledge on the aetiology of infections caused by anaerobic bacteria has serious epidemiological repercussions and forces local clinicians to prescribe antibiotic therapy empirically when treating these infections. Moreover, clinicians often follow the trends and recommendations for developed countries, which may not always apply to the epidemiological characteristics of developing societies.